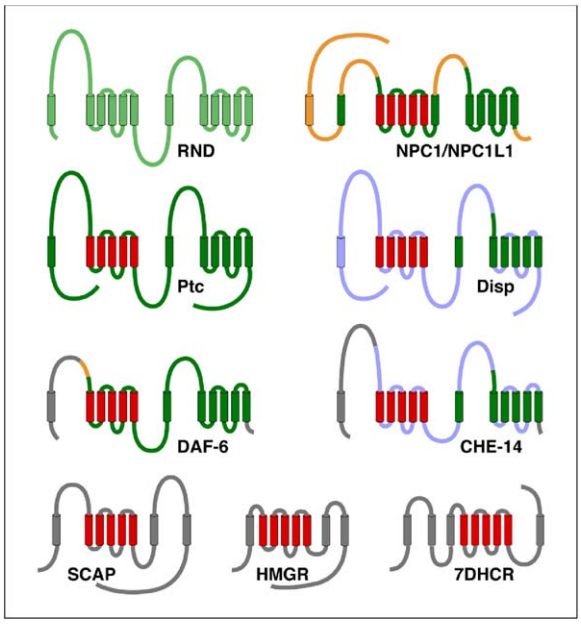


Figure 1. Schematic Structures of RND-SSD Superfamily Members and SSD-Only Proteins

Conserved regions among the most closely related members are color coded and are based on a combination of Clustal W and Blast analyses. Cylinders represent transmembrane (TM) segments with the five SSD segments in red; extramembrane loops are only roughly to scale. A generic representation of an RND family member (light green) is based on the *E. coli* AcrB protein. As the archetypal RND-SSD member, Ptc has weak homology to RND permeases throughout (dark green), including within the SSD. Regions with strong similarity to Ptc are shown in dark green in other superfamily members. Regions unique or strongly similar to NPC1 are in gold, and those unique or similar to Disp are in lavender. Regions with no significant similarities are in gray. Abbreviations not in the text are HMGR, 3-hydroxymethyl-3-glutaryl coenzyme A reductase; 7DHCR, 7-dehydrocholesterol reductase; and SCAP, sterol response element binding protein (SREBP)-cleavage activating protein.

may function in bulk membrane transport. Perens and Shaham favor a model in which DAF-6 and CHE-14 promote deposition of membrane as the lumen grows. However, the cell-surface localization observed for PTC-1, DAF-6, and CHE-14 suggests other models. NPC1 is exclusively endosomal, and although fly and vertebrate Ptc can be detected on the cell surface biochemically, standard imaging techniques reveal only vesicular signal. One situation where stable surface localization of Ptc was imaged was when the rate of Ptc internalization was slowed by a transmembrane form of Hh on an adjacent cell (Incardona et al., 2000). Could DAF-6 be stabilized at the cell surface as a receptor for the lumen-forming cue from neuronal cilia? What stabilizes *C. elegans* PTC-1 at the plasma membrane?

So many fundamental questions remain regarding these enigmatic proteins. Are the Ptc-related proteins orphan receptors in need of ligands or binding partners? Are they catalytic or structural? Are all SSDs really sterol or lipid sensors? Are they multimeric (like bona fide RND permeases)? Do they have small molecule substrates or regulators or even require a proton gradient for function? I could go on. Hopefully, study of the multiplicity of *C. elegans* RND-SSD genes will not complicate the situation further but perhaps help identify a unifying theme to this bewildering superfamily.

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# Degrade or Die: A Dual Function for Autophagy in the Plant Immune Response

**Localized programmed cell death (PCD) is part of a widespread defense mechanism in plants. A recent paper in *Cell* (Liu et al., 2005) shows that autophagy, a process in which cytoplasm and sometimes organelles are engulfed by double membrane vesicles and degraded, is essential for preventing uncontrolled local and systemic PCD during infection and for limiting viral replication.**

Plant cells are connected by cytoplasmic bridges (called plasmodesmata) that promote the exchange of signals and by cell wall polymers that prevent cell mi-

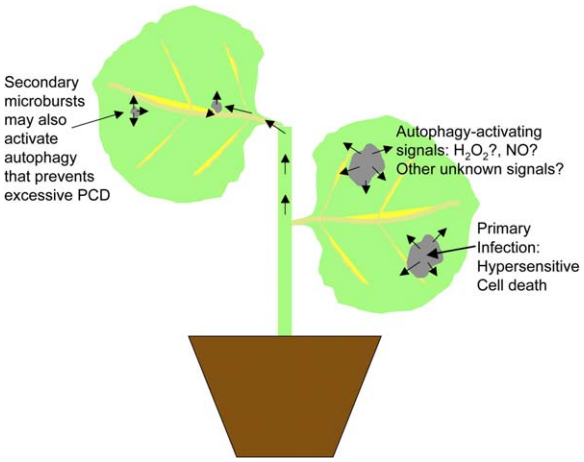


Figure 1. Cell Death Control during the Hypersensitive Response

Primary infection with TMV leads to initial cell deaths that spread a few cell lengths. The extent of cell death spreading depends on reactive oxygen and NO produced in cells surrounding those that initially die. Possibly autophagy is induced by a common signal emanating from infection zones and microbursts in local and systemic tissue, respectively. The activation of autophagy limits the amount of cell death, but the mechanism by which these cells die remains to be investigated.

gration. The consequence during development of this unique architecture is that morphogenesis is achieved by differential division and expansion of cells and by the intercellular trafficking of signals, sometimes as large as mRNAs and proteins, through plasmodesmata. Viruses take advantage of plasmodesmata to spread from cell to cell. The lack of cell migration means that plants lack specialized immune cells. Instead, each plant cell has the ability to defend itself against invaders. Added to this cell-autonomous defense capacity are a number of systemic inducible responses that prime plants to be resistant to subsequent infections.

A prominent defense mechanism against many types of pathogens is called the hypersensitive response (HR), a type of programmed cell death (PCD). The HR is genetically controlled: only when a plant has the specific genotype that allows it to recognize a specific isolate of a pathogen will the response occur. When the HR is disrupted by downregulation of a vacuolar-processing enzyme with caspase activity that is essential for PCD execution, replication of tobacco mosaic virus (TMV) increased (Hatsugai et al., 2004). Thus, the HR restricts viral replication.

Initiating events for the HR result from the perception of specific pathogen-derived molecules. An oxidative burst, ion channels, and/or nitric oxide have been implicated in control of the HR. Nitric oxide and hydrogen peroxide may not be causal for the very first cell death events during infection, but instead may contribute to the death of cells neighboring those that die first (Greenberg and Yao, 2004). This implies that there are multiple signals that stimulate cell death within the zone of cells that die during the HR. It is reasonable to think that plants also have mechanisms to limit the number of cells that die during the HR to prevent excessive tissue loss. Some of these mechanisms have been uncovered through the study of mutants with unrestricted cell death spreading. These studies have pointed to the importance of the LSD1/LOL proteins in redox sensing (Greenberg and Yao, 2004) and ACD2 in the removal of PCD-inducing porphyrin-related molecules that likely are liberated by dying cells (Yao et al., 2004). A recent paper in *Cell* (Liu et al., 2005) now shows that a process called autophagy also plays a prominent role in controlling the spread of cell death during TMV-induced HR.

Macroautophagy (hereafter referred to as autophagy) is a conserved process in eukaryotes in which double membrane vesicles engulf cytoplasm and sometimes organelles. These vesicles are then acidified and targeted for lysosomes or vacuoles, depending on the organism. The contents of the vesicles are then degraded. In plants, mutations that block autophagy result in premature senescence (Thompson and Vierstra, 2005). In yeast and mammalian cells, nutrient starvation also leads to autophagy. Liu et al. (2005) have shown using viral-induced silencing in the model plant *Nicotiana benthamiana* that several genes important for autophagy are important for preventing uncontrolled cell death. Remarkably, plants silenced for any one of several autophagy components show not only spreading cell death at TMV infection sites, but also systemic cell death. This systemic death is not due to spread of the virus. It is possible to recapitulate these effects on cell death by using just the viral protein p50 that elicits the HR. Liu et al. (2005) also show evidence that autophagosomes form adjacent to the infection zone and in systemic tissue. It's likely that the induction of autophagy is not restricted to viral infections, as HR elicitors from fungi and bacteria cause unrestricted cell death in plants silenced for autophagy components. There appears to be some specificity to the role of autophagy, however, since the death of tissue due to methanol exposure does not lead to cell death spreading when autophagy genes are silenced.

Liu et al. (2005) also showed that blocking autophagy results in increased local viral replication. This is perhaps not surprising, since the autophagic pathway has been implicated as antiviral in mammalian cells. Presumably, this is due to the engulfment of virus particles into autophagosomes (Kirkegaard et al., 2004), but this has not been clearly demonstrated in the plant system. What is surprising is that suppression of autophagy leads to local and systemic cell death during pathogen attack. There is ample evidence in mammalian cells for autophagy being associated with a process called type 2 cell death (Clarke, 1990). However, there are clues that activation of cell death in response to blocking autophagy might be a conserved phenomenon. In human cells, nutrient starvation or treatment with chemicals that induce autophagy leads to apoptosis when autophagy is blocked (Boya et al., 2005). This implies that autophagy suppresses apoptosis possibly in both plants

and humans. It should be noted, however, that the current work in plants did not explore the mechanism by which the cells died when autophagy was suppressed.

Key questions to be answered are as follows. What is the signal that induces autophagy during infection? Why does blocking autophagy lead to systemic cell death? The answers to these questions might be related (see Figure 1). The HR generates high levels of reactive oxygen and nitric oxide in the infection zone. Additionally, a local HR was found to induce systemic "microbursts" that also generate reactive oxygen and result in small clusters of cell deaths in uninfected regions (Alvarez et al., 1998). Whether these microbursts also involve nitric oxide is unknown. If reactive oxygen (and/or nitric oxide) is an autophagy trigger, then it's possible that when autophagy is blocked, the reactive oxygen/nitric oxide leads to increased cell death. It is intriguing that low level of oxidative stress induces a form of chaperone-mediated autophagy in mammalian cells that is thought to be important for removing oxidatively damaged proteins (Kiffin et al., 2004). It will be interesting to determine whether macroautophagy also has a relationship with oxidative stress in mammalian and/or plant cells. An alternative hypothesis is that autophagy generally modulates the balance of pro-PCD and anti-PCD molecules. New tools that allow nondestructive in vivo monitoring of autophagy and the redox status of cells should help resolve these questions.

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